

Phase diagram of mechanically stretched DNA: The salt effect

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Abstract

The cations, in form of salt, present in the solution containing DNA play a crucial role in the opening of two strands of DNA. We use a simple non linear model and investigate the role of these cations on the mechanical unzipping of DNA. The Hamiltonian is modified to incorporate the solvent effect and the cations present in the solution. We calculate the melting temperature as well as the critical force that is required to unzip the DNA molecule as a function of salt concentration of the solution. The phase diagrams are found to be in close agreement with the experimental phase diagrams.

1 Introduction

The stability of the double stranded DNA (dsDNA) molecule is primarily due to the hydrogen bonding present between the bases of the complimentary strand. The bases along the strands give rise to the stacking interaction between the nearest base pairs which contributes to the rigidity of the molecule. In addition to this, the presence of the cations (Na^+ or Mg^{2+}) in the form of salt (in the solution) plays a crucial role in the stability of these molecules. The stability of the strand can be monitored by changing the temperature, by applying the force on either of the ends or by changing the pH of the solution. A systematic investigation done in the past have shown that the melting temperature of a dsDNA increases with the salt concentration [1, 2, 3, 4, 5, 6, 7, 8]. Since the two strands of the dsDNA are negatively charged, to neutralize the Coulombic repulsion between the phosphates, the cations like sodium or magnesium ions are required. The concentration of these ions contribute not only to the stability of the molecule but also play an important role in the folding kinetics of the molecule. To understand the mechanism theoretically, the counterion condensation model (based on the two state ion distribution) [9], the Poisson-Boltzmann model (based on mean field calculations) [10, 11] have been used. Recently, the tight bonding approximation (TBA) [12], Poland-Scheraga (PS) [13, 14] and Peyrard Bishop Dauxious (PBD) [3] models are also used to study the helix coil transition in these molecules. Most of these studies focused primarily on the thermal stability of the dsDNA molecule as a function of salt concentration of the solution.

In the recent years, using single molecule force spectroscopy (SMFS) experiments *e.g.* optical & magnetic tweezers, atomic force microscope etc. the forces exerted by single stranded binding (SSB) proteins in maintaining the open regions of ssDNA has been measured directly [4, 5, 6, 7]. These groups have experimentally measured the force required to destabilize the dsDNA as a function of concentration of salt in the solution. In addition to this, several groups have also measured the presence of the salt on the stretching behavior of DNA [15, 16, 17, 18]. All these experimental results provide the measurement of these forces as a function

of salt concentration. However, the theoretical understanding of these results is also important in order to get the precise idea of the physical processes that are involved in these transition. In this manuscript, we investigate the effect of salt present in the solution, on the force induced unzipping of a heterogeneous dsDNA molecule using PBD model [19], which has been discussed in section 2. In this section, we also discuss the method to calculate the melting temperature (T_m) and the forces required to unzip the chain. The method developed in section 2, has been extended to study the thermal & force induced melting of dsDNA in section 3 & 4, respectively. Section 5 summarizes the results followed by brief conclusions.

2 The model

In this section, we briefly discuss the basic features of the PBD model, which considers the stretching between corresponding bases only. Unlike the PS model, which is based on the two state model (bound segment or unbound segment), the PBD model includes intermediate state because the stretching is a continuously varying variable. Although the model ignores the helicoidal structure [20, 21, 22, 23, 24] of the dsDNA molecule. It has enough details to analyze mechanical behavior at few Å scale relevant to molecular-biological events. The Hamiltonian for the considered system of N base pairs unit is written as,

$$H = \sum_{i=1}^N \left[\frac{p_i^2}{2m} + V_S(y_i, y_{i+1}) + V_M(y_i) + V_{\text{sol}}(y_i) \right] \quad (1)$$

where y_i represents the stretching from the equilibrium position of the hydrogen bonds, $p_i = m\dot{y}_i$ represents the momentum while m is the reduced mass of a base pair (taken to be the same for both A-T and G-C base pairs). The stacking interaction between two consecutive base pairs along the chain is represented by,

$$V_S(y_i, y_{i+1}) = \frac{k}{2}(y_i - y_{i+1})^2[1 + \rho e^{-b(y_i + y_{i+1})}], \quad (2)$$

where k represents the single strand elasticity. The anharmonicity in the strand elasticity is represented by ρ while b represents its range. These parameters are assumed to be independent of sequence heterogeneity. The sequence heterogeneity has effect on the stacking interaction along the strand. This can be taken care through the single strand elasticity parameter k .

The hydrogen bonding between the two bases in the i^{th} pair is represented by the Morse potential,

$$V_M(y_i) = D_i(e^{-a_i y_i} - 1)^2, \quad (3)$$

where D_i represents the potential depth, roughly equal to the bond energy of that pair and a_i represents the inverse of the width of the potential well. The heterogeneity in the sequence is taken care by the values of D_i and a_i . In the stability of the dsDNA molecule the role of hydrogen bond is the key factor. In most of the previous studies, the hydrogen bond interaction and the effects of surroundings, such as salt concentration of the solution, are taken as constant [19, 20]. As the DNA molecules are strong polyelectrolytes, having negatively charged phosphate groups, it would be interesting to analyze its role in the melting or unzipping profiles. The salts present in the solution neutralize the negative charge of the phosphate groups, therefore, the increase in their concentration will reduces the electrostatic repulsive forces between these negatively charged groups. Since system at higher concentration prefers to be in less entropic state, more thermal or mechanical energy will be required to break the hydrogen bonds. In the PBD model, the stability in hydrogen

bond is represented by the depth of Morse potential, D_i . Thus, this parameter should be a function of salt concentration of the solution. Experimental observations predict that the melting temperature of dsDNA scales logarithmically with the salt present in the solution[2, 25]. In addition to this, the melting temperature has been found to have a linear dependence on the value of potential depth. Keeping these factors in the background, we modify the potential depth as,

$$D_i = D_0 \left[1 + \lambda \ln \left(\frac{C}{C_0} \right) \right] \quad (4)$$

Here, the concentration, C is expressed in moles per liter and C_0 is the reference concentration chosen to be 1 mole/liter. The λ appearing in the potential is a solution constant [3, 26].

An additional term in the Hamiltonian is the solvent term which simulates the formation of hydrogen bonds with the solvent, once the hydrogen bonds are stretched by more than their equilibrium values. We adopts the solvent term from the refs. [27, 28].

$$V_{sol}(y_i) = -\frac{1}{4}D_i [\tanh(\gamma y_i) - 1] \quad (5)$$

The “tanh” term in the potential enhances the energy of the equilibrium configuration and the height of the barrier below which the base pair is closed. The small barrier basically determines the threshold stretching of hydrogen bond about which a base pair may be temporarily broken, re-bonded and then fully broken. Of course, this come to the broken state at a length greater than $\sim 2\text{\AA}$. As solvent role is to stabilize the denatured state, this form of potential can be a good choice. The term, γ is the solvent interaction factor and it reduces the height of the barrier appears in the potential [27, 28, 29, 30]. We tune various values of γ from 0.1 to 1.0 and plotted the effective potential, as shown in figure 1 B. We found that for larger values of γ , unzipping transition is more favorable. As the broken state is stabilized by the solvent, the transition becomes easier, which reflects the experimental observations.

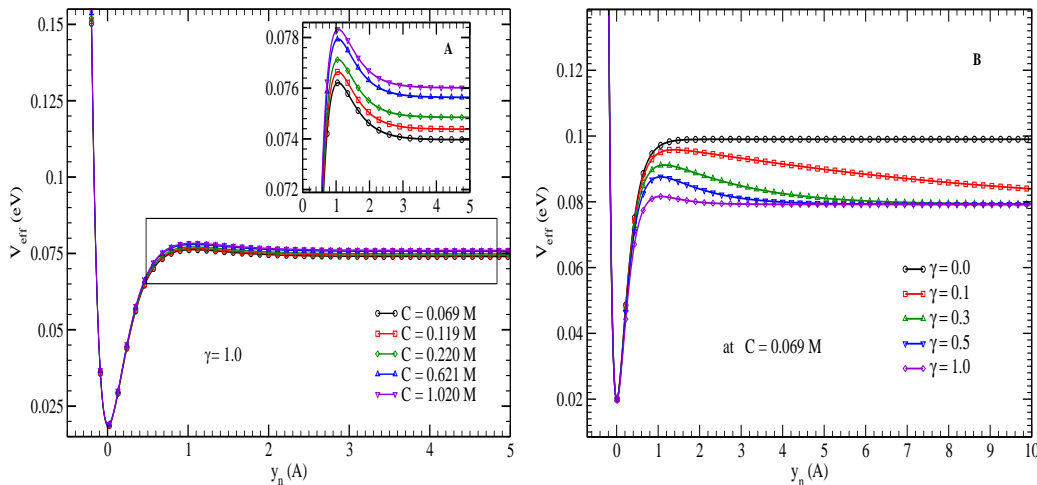


Figure 1: Plot of effective potential $V_{\text{eff}} = V_M + V_{\text{sol}}$ as a function of base pair stretching (in Å). In figure A, the effect of increase in the salt concentration of the solution on the potential depth is shown. The variation in the barrier height with the increase in the solvent interaction factor γ is shown in figure B.

Thermodynamics of the transition can be investigated by evaluating the expression for the partition

function. The canonical partition function is written as,

$$Z = \int \prod_{i=1}^N \{dy_i dp_i \exp[-\beta H(y_i, y_{i+1})]\} = Z_p Z_c, \quad (6)$$

where Z_p corresponds to the momentum part of the partition function while the Z_c contributes as the configurational part of the partition function. Since the momentum part is decoupled in the integration, it can be integrated out as a simple Gaussian integral. This will contribute a factor of $(2\pi m k_B T)^{N/2}$ in the partition function, where N is the number of base pairs in the chain. The configurational partition function, Z_c , is defined as [31],

$$Z_c = \int \prod_{i=1}^N dy_i K(y_i, y_{i+1}) \quad (7)$$

where $K(y_i, y_{i+1}) = \exp[-\beta H(y_i, y_{i+1})]$. For the homogeneous chain, one can evaluate the partition function by transfer integral (TI) method by applying the periodic boundary condition. In case of heterogeneous chain, with open boundary, the configurational part of the partition function can be integrated numerically with the help of matrix multiplication method. Once the limit of integration has been chosen, the task is reduced to discretized the space to evaluate the integral numerically. We choose the limits as -5.0 \AA to 200.0 \AA , as the lower and upper limits of the integration, respectively. The space is being discretized using the Gaussian quadrature formula with number of grid points equal to 900. In our previous studies [31], we observed that to get precise value of melting temperature (T_m) one has to choose the large grid points. We found that 900 is quite sufficient number for this purpose. As all matrices in eq.7 are identical in nature the multiplication is done very efficiently. The thermodynamic quantities of interest can be calculated by evaluating the Helmholtz free energy of the system. The free energy per base pair is,

$$f(T) = -\frac{1}{2} k_B T \ln(2\pi m k_B T) - \frac{k_B T}{N} \ln Z_c. \quad (8)$$

The other thermodynamic quantities like specific heat (C_v) is evaluated using the following relations,

$$C_v(T) = -T \frac{\partial^2 f}{\partial T^2}. \quad (9)$$

We also monitor the fraction of open pairs as a function of temperature and force. The details of process of separation of short chains are different from that of the long dsDNA chains. For long chains, when the fraction ϕ of open base pairs goes practically from 0 to 1 at the melting transition, the two strands are not yet completely separated. At this point, the great majority of the bonds is disrupted and the dsDNA has denaturated, but the few bonds still remain intact, prevent the two strands going apart from each other. The real separation occurs only at high temperatures. For very long chains, the double strand is always a single macromolecule, and hence one need to calculate the fraction of intact or broken base pairs only.

The situation is, however, more involved for short chains. In case of short chains, the end entropy contributes significantly in addition to the loop entropies. Hence the breaking of few bonds as well as the strand separation happens to be in a very narrow range of temperature. Thus average fraction $\theta (= 1 - \phi)$ of bonded base pairs is defined as [32, 33, 34],

$$\theta = \theta_{ext} \theta_{int} \quad (10)$$

θ_{ext} is the average fraction of strands forming duplexes, while θ_{int} is the average fraction of unbroken bonds in the duplexes. The equilibrium dissociation of the duplex C_2 to single strand C_1 may be represented by

the relation $C_2 = 2C_1$. The dissociation equilibrium can be neglected in the case of long chains; while θ_{int} and thus θ goes to zero while θ_{ext} is still practically 1. As discussed above, when θ goes practically from 1 to 0 at the melting, the two strands may not get completely separated, while for short chains, the single bond disruption and strand dissociation occur in a very narrow range of temperature. Therefore, one need to compute both θ_{int} and θ_{ext} .

To compute θ_{int} , one has to separate the configurations describing a double strand on the one hand, and dissociated single strand on the other [33]. Since a bond between two bases is said to be broken if their separation is greater than the average separation between two bases. Therefore, i^{th} bond is considered to be broken if the value of y_i is larger than a chosen threshold y_0 . A configuration belongs to the double strands if at least one of the y_i^s is smaller than this y_0 . One can therefore define θ_{int} for an N base pair duplexes by:

$$\theta_{int} = \frac{1}{N} \sum_{n=1}^N \langle \vartheta(y_0 - y_i) \rangle \quad (11)$$

where $\vartheta(y)$ is Heaviside step function and the canonical average $\langle . \rangle$ is defined considering only the double strand configurations. For y_0 , we have taken a value of 2 Å.

For θ_{ext} we use the expression given in refs. [33, 34].

3 Temperature induced transition

When the dsDNA is in a thermal bath, due to thermal fluctuations the individual bonds may disrupted. This cause the thermal melting of dsDNA. In this section we investigate the role of salt concentration on the thermal stability of the dsDNA molecule. For most of the thermal denaturation studies this effect has been ignored. Here we extend the previous studies on thermal denaturation of dsDNA using modified PBD model and reproduce the experimental findings. We choose three chains, for which the experimental results are available [2]. These chains vary in terms of the fraction of GC & AT base pairs. We call them as 30% GC, 50% GC and 75% GC chains. The chains are,

- (a) 5'-TGATTCTACCTATGTGATTT-3' (30% GC)
- (b) 5'-TACTTCCAGTGCTCAGCGTA-3' (50% GC)
- (c) 5'-GTGGTGGGCCGTGCGCTCTG-3' (75% GC)

As the number of base pairs in all the three chains are 20, we consider them as short chains. We adjust the model parameters to match our results with the experiment [2]. It is found that the stiffness of the chain plays a crucial role in the melting or denaturation of the dsDNA molecule, in addition to the bond energy. While bond energy is represented by Morse potential, the strand elasticity is represented by stiffness parameter k in the anharmonic stacking term. Thus along with the value of D , we check various values of k and ρ to get close match the experimental results. We found the values of $\rho = 1.0$ and $\kappa = 0.01$ eV/Å² as suitable choice for the current investigation.

To adjust the melting temperature within the range of the experimental observations, we finally tune the values of D , keeping other parameters same for all the three chains. With the value of D (for AT base pair) as 0.090, 0.089 & 0.087 eV for 30% GC, 50% GC & 75% GC chain respectively, we found close match with the experimental results for all the concentrations. The values of D for GC base pair is 1.5 times of these values. In the present investigation the stiffness parameters is considered as site independent, however, elasticity of the strand is suppose to depend on the distribution of different bases along the strand [8, 25]. The effect

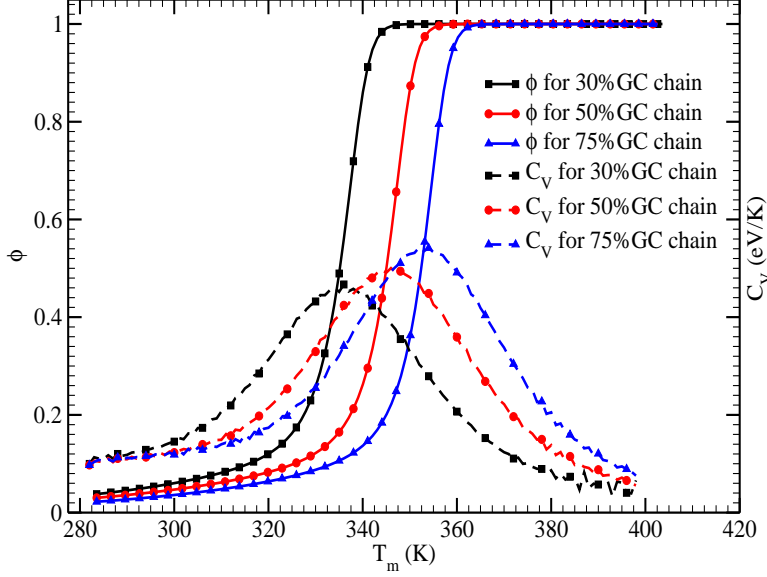


Figure 2: The average number of open pairs as calculated using eq.(10) for salt concentration of 0.621 M for all the three chains. As these are short chains, we calculate $\theta = \theta_{ext}\theta_{int}$. The value of C_v is scaled to show that the peak position and the 50% of the open pairs meet at the same point (temperature).

of the stacking heterogeneities are left for further investigations. The complete set of model parameters (except potential depth D) is: the inverse of potential depth, $a_{AT} = 4.2 \text{ \AA}^{-1}$, $a_{GC} = 6.3 \text{ \AA}^{-1}$, single strand elasticity, $\kappa = 0.01 \text{ eV/\AA}^2$, anharmonicity in the strand, $\rho = 1.0$, range of anharmonicity, $b = 0.35 \text{ \AA}^{-1}$, solution constant, $\lambda = 0.01$ and the solvent interaction factor $\gamma = 1.0 \text{ \AA}^{-1}$.

We calculate the free energy and the specific heat per base pair of the system using eq. (8) and eq. (9). At the temperature when the system gets the sufficient amount of energy that is needed for transition from double stranded configuration to single stranded configuration the free energy shows a kink. For better visualization, we show the transition through the specific heat per base pair as a function of temperature. At the transition point, this is shown by a peak. In order to avoid the overflow we show the curve in fig. 2 for only one value of concentration (0.621 M) for all the three chains. We also monitor the fraction of open pairs as a function of temperature using eq. (10). For short chains, the breaking of hydrogen bonds and the strand dissociation occurs in the same temperature range, thus, one has to calculate the θ_{ext} as well as θ_{int} [33]. The chain is said to be denatured when 50% of the base pairs are in open state. The temperature corresponding to $\theta = 0.5$ is same as we get from the calculation of specific heat (fig. 2). Although the nature of all the three curves are different, they show the transition from double stranded configuration to single stranded configuration of DNA molecule. We obtain the value of melting temperature, T_m , for all the five concentrations for which the experimental results are available. The results are shown in fig. 3. The phase diagram in fig. 3, shows the variation in the melting temperature as a function of salt concentration of the solution. It has been observed, experimentally as well as theoretically, that the melting temperature has a logarithmic dependence on the concentration of Na^+ in the solution. The value of potential depth, D , is tuned to get the proper match with the experimental data. The results shown are close to the experiments, however at low value of salt concentration our data points have slight deviation ($\sim 2\text{-}3 \text{ K}$) from the experimental

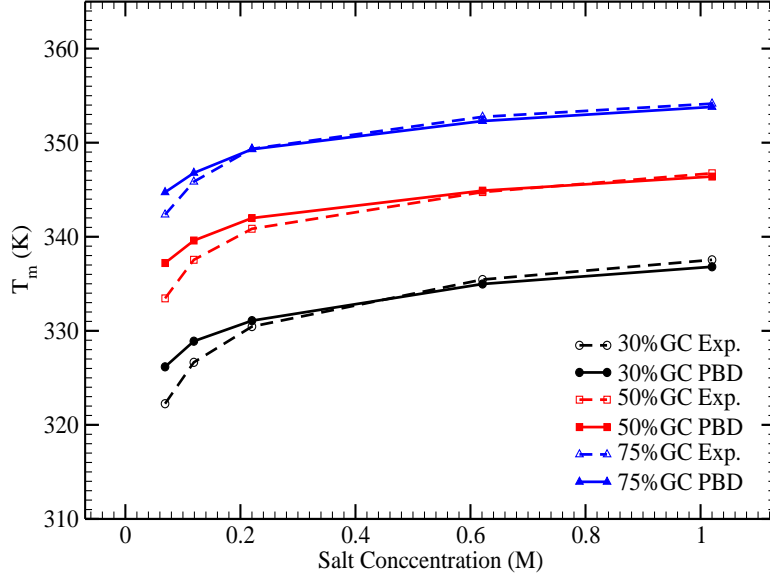


Figure 3: Temperature-salt phase diagram showing the variation in T_m as a function of salt concentration for all the three chains. The melting temperatures are evaluated at the concentrations, 0.069 M, 0.119 M, 0.220 M, 0.621 M & 1.02 M as the experimental data for these values are available [2].

data. We observe that the deviation from the experimental data at low concentration, is most for weaker chain (30% GC). At the lower concentration the melting of AT & GC pairs differs significantly [25] and the stiffness parameter may play an important role. Further investigation is required to explore the stacking energy as a function of the salt concentration in order to get closer match with the experimental results at the lower concentrations .

4 Force induced transition

In this section we investigate the role of salt concentration on the mechanical unzipping of dsDNA molecule. In *vitro*, the double stranded DNA is pulled mechanically, keeping other end fixed. These experiments are performed either at constant displacement of end pairs [31, 35] and calculating the force required by the derivative of the work done in the process or at varying loading rates [36]. Although both the set-ups give the same critical force for an infinite chain, the microscopic and dynamic behavior of unzipping of dsDNA in the the two ensembles are different. When the displacement is held constant, the force adjusts to compensate for the different average binding energies in AT-rich and GC-rich regions. This ensemble known as constant extension ensemble (CEE). The force required to break a pair fluctuate around the value of critical force, F_c . In this case, the large jumps and metastable states are usually absent. In the other ensemble that is constant force ensemble (CFE), the dsDNA is unzipped by applying constant force on one end of the strend keeping other end fixed. For homopolymeric DNA, the unzipping transition is smooth with the constant applied rate, once the constant applied force exceeds the threshold for separating the single base pairs. However, for heterogeneous chain, the transition from double stranded to single stranded is not smooth, but having several pauses and jumps depending on the distribution of weak (AT) and strong (GC) pairs. In the current

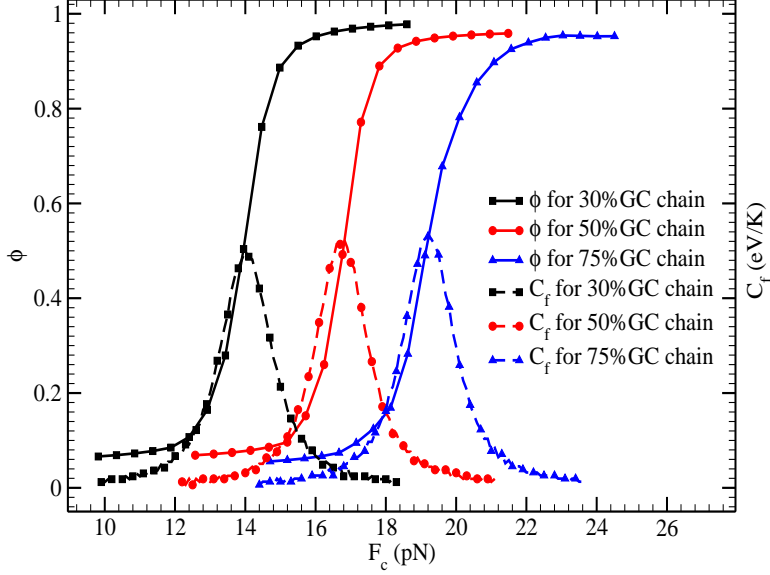


Figure 4: The average number of open pairs as a function of the applied force on the chain for the salt concentration of 0.621 M. As this is for an infinite chain, the $\theta \approx \theta_{int}$. Again value of specific heat is scaled to get the two curves on the same plot.

investigation we calculate the unzipping force in the constant force ensemble [37, 38]. We take the same sequence of 20 base pairs which we considered for thermal studies, however we repeat them to a length which can be considered as an infinite chain. We found that a length of about 600 base pairs is sufficient to be considered as an infinite chain. This length may be model dependent and one may get different number of base pairs in a chain that can be considered as infinite chain. The modified Hamiltonian of the system is,

$$H_f = H - F \cdot y_e \quad (12)$$

We include a term $F \cdot y_e$ in eq. (12), as the force is applied on the end pair. The other model parameters are taken same as in the previous section, however, we tune the value of potential depth D in such a way that we get closer to the experimental results [6] for 50% GC chain. We take the value of potential depth as $D_{AT} = 0.076$ eV for all the three chains. As the force induced unzipping experiments are performed at room temperature (~ 300 K), the melting temperature T_m of the system should be much higher than 300 K, in order to ensure that at 300 K, base-pair opening is not due to thermal fluctuations. With these sets of model parameters T_m is approximate 350 K for 50% GC chain. We calculate the free energy of the system as a function of applied force. The force is applied on the 3' end of the chains at temperature 300 K. At the critical force we observe a kink in the free energy which gives rise to the peak in the specific heat at constant force as shown in fig. 4. This is the point where the system transform from close state to open state. We obtain the value of critical force for all the three chains by locating the peak in specific heat as a function of applied force. We also calculate the average number of open pairs in a chain as a function of the applied force. From the fig. 4, it is clear that the $\phi = 0.5$ at the same value of force as predicted by specific heat as a function of applied force. As the calculation for force induced unzipping is for infinite chain, we calculate the $\phi = 1 - \theta = 1 - \theta_{int}$.

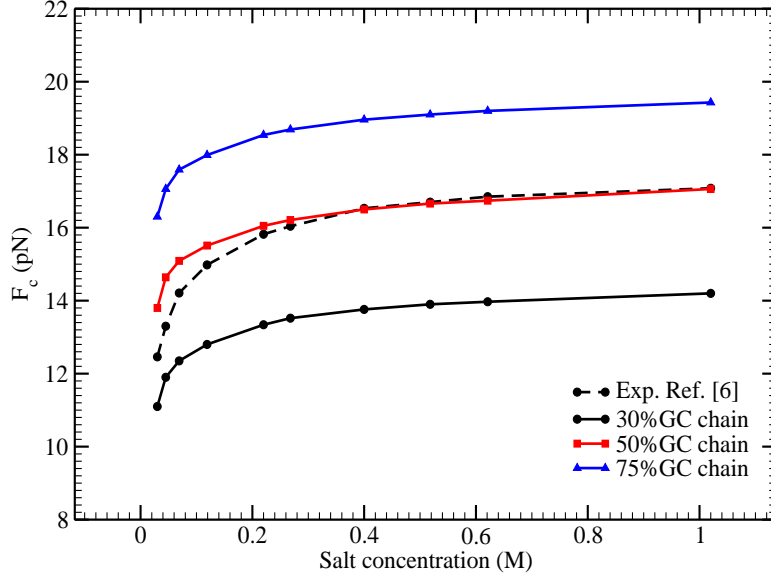


Figure 5: The phase diagram showing the dependence of critical force on the salt concentration of the solution. For comparison we show the experimental results obtained by [6], in the diagram.

The phase diagram in fig. 5, shows the two phases in case when the dsDNA is forced to unzip mechanically as a function of salt concentration. We compare our results with the experimental phase diagram [6]. The results reported here are in good match with the experimental results except for low concentrations, where the slight deviation has been observed (~ 0.5 - 1.4 pN). As discussed earlier the stacking interaction contributes significantly at lower salt concentration during forced induced unzipping of DNA chain.

5 Conclusions

In this manuscript, we have investigated the role of salt concentration on the thermal as well as on the mechanical unzipping behavior of heterogeneous dsDNA molecule. The PBD model is modified to incorporate the salt as well as the solvent effect of the system. Our results indicate a close match with the earlier observations on thermal denaturation of dsDNA, which shows that the melting temperature varies non-linearly or logarithmically with the salt concentration of the solution. As predicted by Manning's counterion condensation theory [9], this is due to the layer of condensed counterions on the DNA surface that neutralizes the phosphate charges. This decreases the inter-strand electrostatic repulsion and the overall stability of DNA molecule increases and hence system need more thermal energy to break or denature. The melting temperature is found to vary with the salt concentration as well as with the GC content of the chain. We have investigated the role of salt present in the solution on the mechanical unzipping behavior of the dsDNA molecule in CFE. We found that the critical force (force needed to completely unzipped the molecule) increases with the increase in the salt concentration of the solution. The addition of salt in the solution basically shields the repulsion between the phosphate groups in the dsDNA chain which in result need more force to unzip the chain. Our results are found to be in close agreement with the experimental phase diagram [6]. However, the deviation at the lower salt concentration needs further attention. The stacking

heterogeneity, which has been taken as constant in the current investigation, might be the key factor at low concentration. We conclude that the PBD model, although a quasi one dimensional model in nature, can be a good choice to investigate the presence of salt in the solution and its effect not only on the thermal denaturation of dsDNA chain, but also on the mechanical unzipping of the chain. In future, it would be interesting to study the dynamics of unzipping of dsDNA when the salt present in the solution is considered. However we would like to investigate this effect on the mechanical unzipping in the case when force is not applied on the one of the ends but some where on the middle of the chain, the situation that is more closer to the transcription process.

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